PTEN/P16 INHIBITS INFLAMMATORY RESPONSE AND REDUCES INTESTINAL METAPLASIA IN GASTRIC MUCOSA OF CHRONIC ATROPHIC GASTRITIS AND PREVENTS THE CARCINOGENESIS

Kailiang Chen[1#], Yu Liu[2#], Chaoqun Wang[3*], Jiafang Xu[4*], Jian Gong[5]
[1]Department of Ultrasound, The First Affiliated Hospital of Hainan Medical University, Haikou 570102, China - [2]Department of Breast and Thoracic Oncological Surgery, The First Affiliated Hospital of Hainan Medical University, Haikou 570102, China - [3]Department of Nuclear Medicine, Hainan General Hospital, Hainan Affiliated Hospital of Hainan Medical University, Haikou 570311, China - [4]Reproductive Medicine Center, The First Affiliated Hospital of Hainan Medical University, Haikou 570102, China - [5]Department of Gastroenterology, Taian City Central Hospital, Taian 271000, China
[#]*Contributed equally*

ABSTRACT

Objective: *To investigate the protective roles of phosphate and tension homology deleted on chromsome ten (PTEN)/p16 in gastric cancer (GC) progression by reducing the production of proinflammatory cytokines and intestinal metaplasia (IM) in gastric mucosa of chronic atrophic gastritis (CAG).*

Methods*: Thirty normal persons (normal group), 30 CAG patients with small intestinal metaplasia (SIM) (CAG+SIM group), 30 CAG patients with colonic intestinal metaplasia (CIM) (CAG+CIM group), and 30 GC patients (GC group) were enrolled. The gastric mucosa tissues were collected. The expressions of PTEN, p16, interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), caudal type homeobox transcription factor (CDX) 1 and CDX2 were detected. The correlations of these indexes in GC tissues were analyzed. Moreover, the expressions of PTEN and p16 in GC cell lines were detected. After over-expressed PTEN and p16 in GC cells were constructed successfully, the expression changes of IL-1β, TNF-α, CDX1 and CDX2 were measured, and the roles of PTEN and p16 in GC cell proliferation and tumor growth abilities were analyzed.*

Results*: The expression levels of PTEN and p16 were decreased while IL-1β and TNF-α, CDX1 and CDX2 were increased in gastric mucosa in CAG+SIM, CAG+CIM and GC groups, compared with normal group. The degrees of these decreased and increased tendencies of above genes were associated with the degree of IM and canceration of gastric mucosa. The levels of IL-1β, TNF-α, CDX1 and CDX2 were negatively correlated with PTEN and p16 in GC specimens. Besides, PTEN and p16 expressions were declined in diverse GC cell lines, and the over-expression of PTEN and p16 could restrain the expressions of IL-1β, TNF-α, CDX1 and CDX2 as well as inhibit the GC cell proliferation and tumor growth.*

Conclusion*: PTEN and p16 can inhibit the inflammatory response and reduce the IM in gastric mucosa of CAG and prevent the occurrence of carcinogenesis.*

Keywords: *chronic atrophic gastritis, gastric cancer, PTEN, p16, inflammatory reaction, intestinal metaplasia.*

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Introduction

Gastric cancer (GC), as a primary aggressive malignancy in the alimentary system, is one of the leading factors of cancer related death all over the world[1-3]. With a rapid rise of morbidity over the past 30 years, GC has brought considerable economic burden to the global health-care system[4]. The 5-year survival rate of GC sufferers is still relatively low under the great improvement of iatrotechniques in recent years[5]. Like other malignancies, the aberrant powerful cell proliferation and tumor growth are the main features of GC[6-8]. Recently, growing evidence shows that the chronic atrophic gastritis (CAG) is closely related to GC. CAG is often accompanied by intestinal metaplasia (IM) which is an indicator of gastric mucosal injury and an important epithelial change in CAG. IM is defined as the disappearance of normal gastric mucosal epithelium and its replacement by Paneth cells, goblet cells or absorption cells. IM is divided into small IM (complete IM) and colonic metaplasia (incomplete

IM) according to the method of mucus histochemical staining[9]. Sarnak and Jaber[10] have proposed a pattern of gastric carcinogenesis, the chronic superficial gastritis-CAG-IM-abnormal hyperplasia-cancerization, indicating that gastric carcinogenesis is an accumulation result of multi-gene mutation caused by multi-step evolution. It is important to study the inactivation and abnormal expression of tumor suppressor genes (TSGs) for elucidating the process of occurrence and development of GC[11].

The TSGs of phosphate and tension homology deleted on chromsome ten (PTEN) and p16 (also named MTS (multiple tumor suppressor 1) are involved in multifold malignancies including neck squamous cell carcinoma[12], canine osteosarcoma[13], GC[14], etc. Besides, PTEN and p16 also participate in regulating the inflammatory response[15,16]. Weightily, inflammation is an important factor in the pathogenesis in multifarious gastrointestinal disorders by influencing the mucosal architecture[17]. However, no literature about the effects of PTEN/p16 on IM is reported. Therefore, this study explored whether the tumor inhibitor gene PTEN/p16 played the roles in GC through the pathways of inflammatory reaction and IM.

Materials and methods

Clinical specimens

Gastric mucosal specimens were acquired from 30 normal persons (18 males and 12 females; 48-62 years, average 55.32±7.87 years) who attended physical examination (normal group), 30 chronic atrophic gastritis (CAG) patients with small intestinal metaplasia (SIM) (16 males and 14 females; 45-63 years, average 54.53±7.27 years) (CAG+SIM group), 30 CAG patients with colonic intestinal metaplasia (CIM) (16 males and 14 females; 49-65 years, average 55.24±7.65 years) (CAG+CIM group) and 30 GC patients (19 males and 11 females; 50-72 years, average 56.53±8.12 years) (GC group) in our hospital from May 2016 to May 2019. All the patients were firstly diagnosed, and the basic data (age, gender, etc.) had no statistical difference among different groups ($P > 0.05$). Classification of IM was based upon method of histochemical staining of mucus, and the diagnosis of CAG with IM and GC was performed by two senior pathologists, independently, according to blind method. All GC patients underwent surgery excision with no prior anticancer cure including chemotherapy and/or radiotherapy. All patients had complete clinical records. This study was approved by the ethics committee of our hospital, and the informed consent was obtained from the family of patients.

Cell culture

The normal human gastric mucosa epithelial cell line GES-1 and human gastric cancer cell line AGS, 23132/87, KATO3 and HGT1 were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). All cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich, St Louis, MO, USA) or RPMI 1640 (Invitrogen, Shanghai, China) with 10% FBS (Gemini Bioproducts, Calabasas, CA) in a 5% CO2 humidified chamber at 37 °C.

RNA interference and transfection assays

For constructing the over-expressed PTEN and p16 in AGS cells, pcDNA3.0 plasmid vector-PTEN and pcDNA3.0 plasmid vector-p16 were synthesized in Shanghai GenePharma Co., Ltd. (Shanghai, China), then were transfered into AGS cells by using Lipofectamine 2000 Transfection reagent (Thermo Fisher Scientific, Carlsbad, CA) according to manufacturer's instructions. The empty vector was used as the control. Transfection efficiency was assayed by using qRT-PCR.

Real-time quantitative reverse transcription–polymerase chain reaction (qRT-PCR)

qRT-PCR was carried out to determine the mRNA expression levels of TSGs of PTEN and p16, inflammatory factors of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), and the common and iconic protein molecules in IM of caudal type homeobox transcription factor 1/2 (CDX1/2) from clinical specimens and various cells referencing the reported study (18). Briefly, the total RNA was isolated using Trizol reagent (Invitrogen, USA) according to the manufacture's guide. After cDNAs were produced by using high-capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA), cDNAs were amplified utilizing the following primers sequences: PTEN (human): forward, 5'-GCCGTTCGGAGGATTATTCG-3', reverse, 5'-AGAGATGGCAGAAGCTGCTG-3'; p16 (human): forward, 5'-GCTGCCCAAC-GCACCGAATA-3', reverse, 5'-ACCACAGCGTGTCCAGGAA-3'; IL-1β (human): forward, 5´-AACAGGCTGCTCTGGGATTCTCTT-3´, reverse, 5´-TCATTTCACTG-

GCGAGCTCAGGTA-3´; TNF-α (human): forward, 5´-TCTTCTGCCTGCTGCACT-TTGG-3´, reverse, 5´-ATCTCTCAGCTCCACGCCATTG-3´; CDX1 (human): forward, 5'-ACAATCCGGCGGAAATCAG-3', reverse, 5'-TTCACTTTGCGCTCCT-TTGC-3'; CDX2 (human): forward, 5'-CTGGAGCTGGAGAGGAGTTTC-3', reverse, 5'-ATTTTAACCTGCCTCTCAGAGAGC-3'. The human GAPDH was used as an internal control, and the primers sequences of GAPDH (human) were: forward, 5'-ACAGTCAGCCGCATCTTCTT-3', reverse, 5'-GTTAAAAGCAGCCCTGGTGA-3'. The relative quantification was measured by the ΔΔCT method. The PCR assays were performed for three times.

Spearman correlation coefficient analysis

The data of expression levels of PTEN, p16, CDX1, CDX2, IL-1β and TNF-α were gathered and the expression relevance among them was analyzed by using spearman correlation coefficient method.

Cell proliferation assay

A cell counting Kit-8 kit (CCK-8; Beyotime, Shanghai, China) was used to assay the cell proliferation following the manufacturer's instructions. Briefly, AGS cells were seeded in 96-well plates at 5x103 cells/well and incubated with 10 μL CCK-8 solution in 100 μL fresh media for 3 h at 37°C. The optical density was read at an absorbance wavelength of 450 nm at 0 h, 24 h, 48 h and 72 h with a full wavelength microplate analyzer (Molecular Devices). Each result was presented by the mean of triplicate assays.

Tumor growth analysis in vivo

Twelve BALB/C nude mice with 6-weeks-old were purchased from the National Experimental Center (Beijing, China). 1×10^7 AGS cells transfected with pcDNA-PTEN/p16 or empty vector were inoculated under the skin of nude mice. The subcutaneous tumor size was measured at every 7 days during 35 days to plot the tumor growth curve. On the 35th day after injection, the mice were executed by twisting the neck. Then, the tumors were resected and weighed to measure the subcutaneous tumor mass. Animal experiments were carried out strictly in accordance with the agreement approved by the administrative department of animal care/treatment in the laboratory of our hospital. The ethical recognition was provided by our hospital.

Statistical analysis

Statistical analysis was performed with SPSS 16.0 (SPSS, Chicago, IL, USA). Results were expressed as means±standard deviation (SD). To assess statistical differences, quantitative data between two groups were assessed using the independent-samples t-test and Student's t-test, and quantitative data between more than two groups adopted one-way analysis of variance. For all continuous data, the normality tests were performed. The Spearman correlation coefficient analysis was performed for comparing the correlation of quantitative data between two groups. $P < 0.05$ was considered as the statistically significant difference.

Results

Expressions of PTEN/p16, IL-1β/TNF-α, and CDX1/CDX2 in clinical specimens

Compared with the normal group, the expressions of PTEN and p16 were decreased in CAG+SIM group ($P < 0.05$), CAG+CIM group ($P < 0.01$), and GC patients ($P < 0.01$), respectively. The PTEN and p16 expression levels were declined in CAG+CIM group compared with CAG+SIM group ($P < 0.05$). Compared with CAG+SIM group, the PTEN and p16 levels were obviously reduced in GC group ($P < 0.01$), and showed a downtrend when compared with CAG+CIM group, but the difference was not statistically significant ($P > 0.05$) (Figure 1 A). Moreover, the expression levels of inflammatory factor IL-1β and TNF-α and symbolic molecules of IM of CDX1 and CDX2 showed a similar opposite trend with PTEN and p16 (Figure 1 B and C).

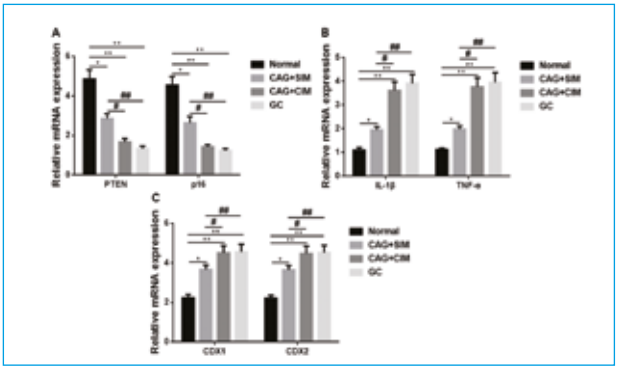

Figure 1: Relative mRNA expressions of PTEN/p16, IL-1β/TNF-α, and CDX1/CDX2 in clinical specimens. PTEN, phosphate and tension homology deleted on chromsome ten; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; CDX1/2, caudal type homeobox transcription factor 1/2. *$P < 0.05$ vs. normal group; **$P < 0.01$ vs. normal group; #$P < 0.05$ vs. CAG+SIM group; ##$P < 0.01$ vs. CAG+SIM group.

Expression relevance between PTEN/p16, IL-1β/TNF-α and CDX1/CDX2 in GC clinical specimens

Spearman correlation coefficient analysis results displayed that, the expression levels of PTEN and p16 had a negative correlation with IL-1β, TNF-α and CDX1 and CDX2 in GC clinical specimens ($P < 0.05$ or $P < 0.01$) (Figure 2).

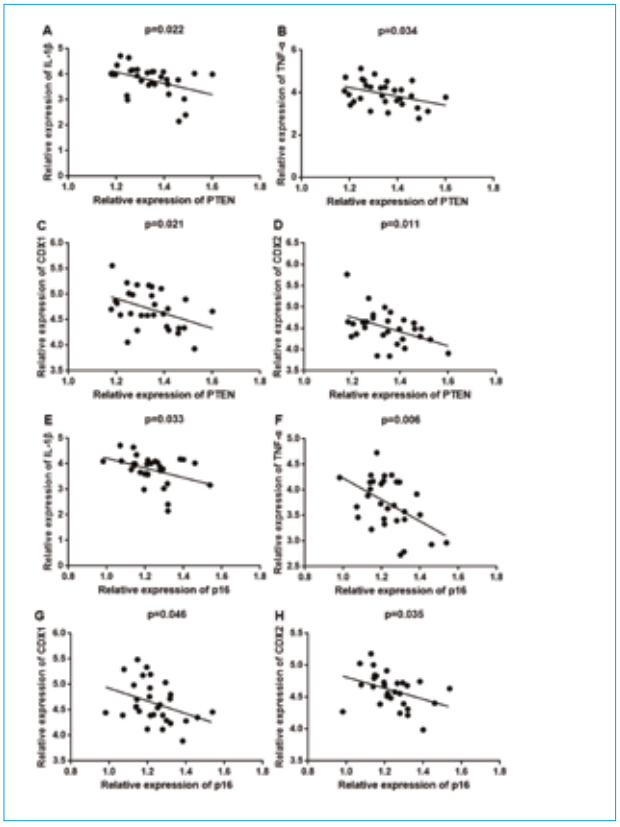

Figure 2: Relevance of relative mRNA expressions between PTEN/p16, IL-1β/TNF-α, and CDX1/CDX2 in GC clinical specimens. PTEN, phosphate and tension homology deleted on chromsome ten; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; CDX1/2, caudal type homeobox transcription factor 1/2; GC, gastric cancer.

PTEN and p16 expression levels were declined in various human GC cell lines

The qRT-PCR results showed that, the relative mRNA expression levels of PTEN and p16 were all reduced in all the enrolled human GC cell lines, particularly in AGS cell line when compared with the normal control GES-1 cell line ($P < 0.05$) (Figure 3). Thereby, the AGS cell line was used as the subsequent experiments in cell and animal in vitro and in vivo, respectively.

Over-expression of PTEN/p16 weakened the inflammatory reaction and IM

Figure 4 A and B revealed that, the over-expressed PTEN and p16 were successfully constructed in AGS cells, respectively. Besides, the over-expression of PTEN/p16 caused a decrease of expressions of IL-1β, TNF-α, CDX1 and CDX2 ($P < 0.05$) (Figure 4 C and D).

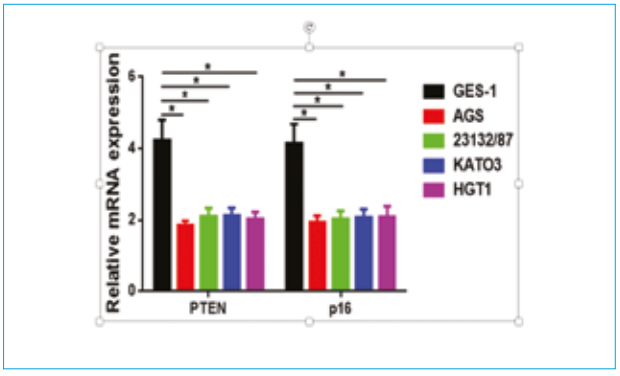

Figure 3: Relative mRNA expressions of PTEN/p16 in various human GC cell lines. *$P < 0.05$ vs. GES-1. PTEN, phosphate and tension homology deleted on chromsome ten; GC, gastric cancer.

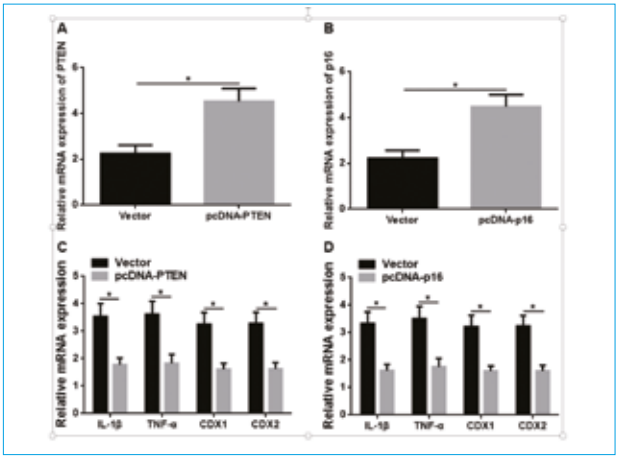

Figure 4: . Over-expression of PTEN/p16 weakened the inflammatory reaction and IM. A and B: Transfection efficiency of the construction of over-expressed PTEN/p16 detected by using qRT-PCR. C and D: Expression changes of IL-1β/TNF-α, and CDX1/CDX2 after up-regulation of PTEN/p16 expression. *$P < 0.05$ vs. vector. PTEN, phosphate and tension homology deleted on chromsome ten; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; CDX1/2, caudal type homeobox transcription factor 1/2; IM, intestinal metaplasia.

Over-expression of PTEN/p16 weakened the occurrence of carcinogenesis

In this study, carcinogenesis could be reflected by malignant biological behaviors of GC, including cell proliferation and tumor growth. The cell proliferation capacity analysis detected by CCK-8 showed that, the over-expression of PTEN and p16 reduced the OD value in 450 nm at 72 h in AGS cells ($P < 0.01$) (Figure 5 A and B). The experiment of subcutaneous tumorigenesis in nude mice showed that, the over-expression of PTEN and p16 obviously

inhibited the tumor volume on 14, 28 and 35 day after seeding the AGS cells in subcutaneous areas in nude mice ($P < 0.01$) (Figure 5 C and D). On the 35th day after seeding the AGS cells in subcutaneous areas in nude mice, the tumor weight presented a marked decrease in nude mice with over-expression of PTEN and p16, compared with null vector control nude mice ($P < 0.01$) (Figure 5 E and F).

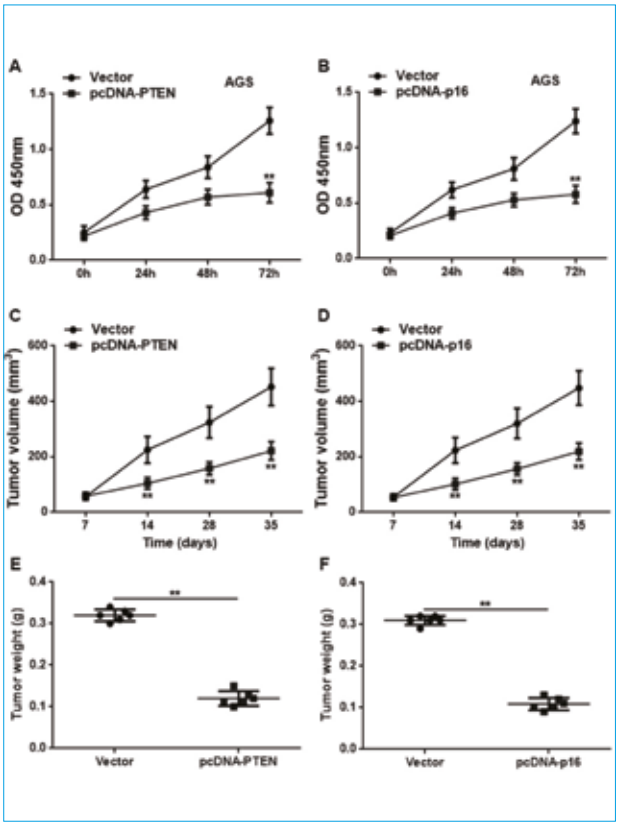

Figure 5: Roles of over-expression of PTEN/p16 in cell proliferation and tumor growth. A and B: Roles of over-expression of PTEN/p16 in cell proliferation reflected by OD values at 450 nm detected by CCK-8. C-F: Roles of over-expression of PTEN/p16 in tumor growth reflected by tumor volume and tumor weight. **P < 0.01 vs. vector. PTEN, phosphate and tension homology deleted on chromsome ten.

Discussion

The initiation and development of GC is a very complex process closely related to a variety of factors involving a genetic and epigenetic changes, especially the activation of oncogenes and the inactivation of TSGs or abnormal expression of them[11,19,20]. Moreover, the loss or inactivation of more than 20 types of TSGs have been reported to be participated in multifarious human solid tumours[21]. Loss of TSG in PTEN expression is closely related to the poor prognosis and outcome, a more advanced stage, lymphatic vessel invasion and nodal metastasis of GC[14,19]. Loss of TSG in p16 expression is associated with the tumour progression both in early and advanced stage of GC[19]. Importantly, CAG is closely associated with GC, and CAG is often accompanied by IM which is usually considered as a precancerous state of GC(22). The current accepted pattern of GC is CAG-gastric mucosal IM-gastric mucosal atypical hyperplasia (intraepithelial neoplasia)-GC. This process is related to polygenic changes including activation of proto-oncogenes and inactivation of TSGs, amplification of proto-oncogene and overexpression of oncogene products[10]. However, the roles of PTEN/p16 on IM have not been reported till now. Therefore, we assume PTEN/p16 can promote the progressions of GC by arousing the inflammatory reaction and IM in gastric mucosa.

In this study, we detected the expressions of TSGs of PTEN and p16, inflammatory factors of IL-1β and TNF-α, and the markers of IM of CDX1 and CDX2 in the gastric mucosa specimens from normal group, CAG+SIM group, CAG+CIM group, and GC group. The results showed that the down-regulated expressions of PTEN and p16, up-regulated expressions of IL-1β and TNF-α, and CDX1 and CDX2 were associated with the degree of IM and canceration of gastric mucosa. Furthermore, the expression levels of IL-1β and TNF-α, and CDX1 and CDX2 were negatively associated with PTEN and p16 in gastric mucosa of human GC. We also found that PTEN and p16 were down-regulated in diverse GC cell lines.Then, over-expressed PTEN and p16 were constructed in GC cells. It is found that the inflammatory reaction and IM were lightened with declined expressions of IL-1β and TNF-α, and CDX1 and CDX2. Meanwhile, the over-expressed PTEN and p16 visibly inhibited the GC cell proliferation and tumor growth abilities. These findings indicate that down-regulated PTEN and p16 levels may cause an increase of expressions of inflammatory factors and IM markers then to aggravate the canceration.

It is well known that colonic IM may occur on the basis of gradual aggravation of small IM.In this study, we found that the expressions of PTEN and p16 were gradually declined in CAG+SIM group, CAG+CIM group and GC group when compared with the normal group, and the PTEN and p16 expression levels were also declined in CAG+CIM group or GC group, compared with CAG+SIM group. Additionally, the expressions levels of IL-1β and TNF-α, and CDX1 and CDX2 revealed a similar opposite trend with

PTEN and p16. Our results supported that colonic IM was more severe than small IM, and colonic IM may more easily develop to GC. Weightily, inflammation can produce a negative impact on mucosal architecture and then trigger diversiform gastrointestinal diseases[17]. Existing assumptions also can support our findings that the glandular neck stem cells of gastric mucosa have the potential to secrete in many aspects, and it can differentiate into mature epithelial cells of various gastric mucosa at normal conditions. Colorectal metaplasia is more prone to carcinogenesis and becomes intestinal gastric cancer after carcinogenesis. In addition, stem cells differentiate directly into intestinal cells in carcinogenesis, and can also form intestinal gastric cancer. CDX1 and CDX2 are the common and iconic protein molecules of IM, which are recognized as intestinal specific factors and the significantly up-regulated expressions of CDX1 and CDX2 occur in gastric mucosa intestinal epithelial metaplasia. CDX1 and CDX2 can induce undifferentiated cells differentiate into intestinal epithelium, and advance the human stomach to arise IM, epithelial dedifferentiation even cancerization[23,24]. In this study, the expression levels of CDX1 and CDX2 also increased in gastric mucosa specimens from CAG+SIM group, CAG+CIM group, and GC group compared with normal group. At present, the IM in stomach is regarded by some scholars as a precancerous lesion of GC, which occurs in about a quarter of individuals around the world[22,25]. Epidemiological study has found that IM patients are more than 10 times more at risk of developing GC(9). Effective diagnosis and regular control of IM patients has become a hot topic in early screening and prevention of GC. Therefore, ameliorate inflammatory reaction and IM may be effective strategies to prevent the development of GC.In the present study, when PTEN and p16 expression was increased, then the cell proliferation and tumor growth abilities of GC cells were dramatically weakened, and the proinflammatory factor levels of IL-1β and TNF-α as well as the labeled molecules of IM of CDX1 and CDX2 were also distinctly declined. Previous researches suggest that aberrant powerful cell proliferation and tumor growth abilities are the main features of GC which can be regulated by oncogenic or anti-oncogenic genes[6-8]. Moreover, as anti-oncogenic genes, PTEN and p16 have been attested to play an inhibitory action on inflammatory response[15,16]. These evidences are consistent with our findings, and our research is the first study to illuminate the inhibitory effects of PTEN and p16 on IM.

To sum up, PTEN and p16 can inhibit the inflammatory response and reduce the IM in gastric mucosa of CAG, thus preventing the occurrence of carcinogenesis. The over-expression of PTEN and p16 may be an effective means of GC. However, this needs to be further verified by more research.

References

1) Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.
2) Liu L, Tian YC, Mao G, Zhang YG, Han L. MiR-675 is frequently overexpressed in gastric cancer and enhances cell proliferation and invasion via targeting a potent anti-tumor gene PITX1. Cell Signal 2019; 62: 109352.
3) Hevia MJ, Castro P, Pinto K, Reyna-Jeldes M, Rodríguez-Tirado F, Robles-Planells C, Ramírez-Rivera S, Madariaga JA, Gutierrez F, López J, Barra M, De la Fuente-Ortega E, Bernal G, Coddou C. Differential effects of purinergic signaling in gastric cancer-derived cells through P2Y and P2X receptors. Front Pharmacol 2019; 10: 612.
4) Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
5) Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, Stein KD, Alteri R, Jemal A. Cancer treatment and survivorship statistics, 2016. CA Cancer J Clin 2016; 66: 271-289.
6) Li H, Wang Y. Long Noncoding RNA (lncRNA) MIR22HG suppresses gastric cancer progression through attenuating NOTCH2 signaling. Med Sci Monit 2019; 25: 656-665.
7) Wei GH, Wang X. lncRNA MEG3 inhibit proliferation and metastasis of gastric cancer via p53 signaling pathway. Eur Rev Med Pharmacol Sci 2017; 21: 3850-3856.
8) Li C, Liang G, Yang S, Sui J, Yao W, Shen X, Zhang Y, Peng H, Hong W, Xu S, Wu W, Ye Y, Zhang Z, Zhang W, Yin L, Pu Y. LncRNA-LOC101928316 contributes to gastric cancer progression through regulating PI3K-Akt-mTOR signaling pathway. Cancer Med. 2019; 8: 4428-4440.
9) Leung WK, Sung JJ. Review article: intestinal metaplasia and gastric carcinogenesis. Aliment Pharmacol Ther 2002; 16: 1209-1216.
10) Sarnak MJ, Jaber BL. Pulmonary infectious mortality among patients with end-stage renal disease. Chest. 2001; 120: 1883-1887.
11) Sharma RK, Sahu KM. Nutrition in dialysis patients. J

Indian Med Assoc. 2001; 99: 206-208, 210-211, 213.
12) da Costa AABA, Costa FD, Araújo DV, Camandaroba MPG, de Jesus VHF, Oliveira A, Alves ACF, Stecca C, Machado L, de Oliveira ACF, de Oliveira TB, Nicolau UR, de Lima VCC. The roles of PTEN, cMET, and p16 in resistance to cetuximab in head and neck squamous cell carcinoma. Med Oncol 2018; 36: 8.
13) Russell DS, Jaworski L, Kisseberth WC. Immunohistochemical detection of p53, PTEN, Rb, and p16 in canine osteosarcoma using tissue microarray. J Vet Diagn Invest 2018; 30: 504-509.
14) Kang HJ, Lee IS, Park YS, Ho WJ, Sohn D, Ahn JY, Yook JH, Kim BS. Biomarkers of EBV-positive gastric cancers: loss of PTEN expression is associated with poor prognosis and nodal metastasis. Ann Surg Oncol. 2016; 23: 3684-3692.
15) Zhang S, He K, Zhou W, Cao J, Jin Z. miR-494-3p regulates lipopolysaccharide-induced inflammatory responses in RAW264.7 cells by targeting PTEN. Mol Med Rep. 2019; 19: 4288-4296.
16) Chikenji TS, Saito Y, Konari N, Nakano M, Mizue Y, Otani M, Fujimiya M. p16INK4A-expressing mesenchymal stromal cells restore the senescence-clearance-regeneration sequence that is impaired in chronic muscle inflammation. EBioMedicine. 2019; 44: 86-97.
17) Cockrell C, Christley S, An G. Investigation of inflammation and tissue patterning in the gut using a Spatially Explicit General-purpose Model of Enteric Tissue (SEGMEnT). PLoS Comput Biol 2014; 10: e1003507.
18) Chen J. miRNA-195 suppresses cell proliferation of ovarian cancer cell by regulating VEGFR2 and AKT signaling pathways. Mol Med Rep 2018; 18: 1666-1673.
19) Lee HS, Lee HK, Kim HS, Yang HK, Kim WH. Tumour suppressor gene expression correlates with gastric cancer prognosis. J Pathol 2003; 200: 39-46.
20) Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990; 61: 759-767.
21) Macleod K. Tumor suppressor genes. Curr Opin Genet Dev 2000; 10: 81-93.
22) Yoon H, Kim N. Diagnosis and management of high risk group for gastric cancer. Gut Liver 2015; 9: 5-17.
23) Jaffee IM, Rahmani M, Singhal MG, Younes M. Expression of the intestinal transcription factor CDX2 in carcinoid tumors is a marker of midgut origin. Arch Pathol Lab Med 2006; 130: 1522-6.
24) Rau TT, Rogler A, Frischauf M, Jung A, Konturek PC, Dimmler A, Faller G, Sehnert B, El-Rifai W, Hartmann A, Voll RE, Schneider-Stock R. Methylation-dependent activation of CDX1 through NF-κB: a link from inflammation to intestinal metaplasia in the human stomach. Am J Pathol 2012; 181: 487-498.
25) Miyake Y, Kobashi H, Yamamoto K. Meta-analysis: the effect of interferon on development of hepatocellular carcinoma in patients with chronic hepatitis B virus infection. J Gastroenterol 2009; 44: 470-475.

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Corresponding Author:
Chaoqun Wang
Department of Nuclear Medicine, Hainan General Hospital, Hainan Affiliated Hospital of Hainan Medical University
31 Longhua Road, Haikou 570311, China
Email: hyxwangcq@163.com
Jiafang Xu
Reproductive Medicine Center, The First Affiliated Hospital of Hainan Medical University,
31 Longhua Road, Haikou 570102, China
Email: xujiafangyuyu@163.com
(China)